

# CLAIMS

1. An isolated nucleic acid segment encoding:
  - a) a polyhydroxyalkanoic acid synthase protein; and
  - b) a fatty acid:acyl-CoA transferase protein.
2. The nucleic acid segment of claim 1, wherein the polyhydroxyalkanoic acid synthase protein is an *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein.
3. The nucleic acid segment of claim 2, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* *phaC* polyhydroxyalkanoic acid synthase structural gene.
4. The nucleic acid segment of claim 1, wherein the fatty acid:acyl-CoA transferase protein is a 4-hydroxybutyrate:acyl-CoA transferase protein.
5. The nucleic acid segment of claim 4, wherein the 4-hydroxybutyrate:acyl-CoA transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein.
6. The nucleic acid segment of claim 5, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein is encoded by the *Clostridium kluyveri* *orfZ* 4-hydroxybutyrate:acyl-CoA transferase structural gene.
7. The nucleic acid segment of claim 1, further comprising a promoter functional in bacterial cells.
8. The nucleic acid segment of claim 1, wherein:
  - a) the sequence encoding a polyhydroxyalkanoic acid synthase protein is operably linked to its native promoter; and
  - b) the sequence encoding a fatty acid:acyl-CoA transferase protein is operably linked to its native promoter.
9. The nucleic acid segment of claim 1, further comprising a promoter heterologous to:
  - a) the sequence encoding a polyhydroxyalkanoic acid synthase protein; and
  - b) the sequence encoding a fatty acid:acyl-CoA transferase protein.
10. A recombinant vector comprising the nucleic acid segment of claim 1.
11. A recombinant vector comprising the nucleic acid segment of claim 9.
12. The recombinant vector of claim 11, further defined as vector pKSSE5.3 wherein: the sequence encoding a polyhydroxyalkanoic acid synthase protein is contained within the 3.5 kb *SmaI*/*Apal* restriction fragment from GenBank Accession number J05003; and

the sequence encoding a fatty acid:acyl-CoA transferase protein is contained within the  
1.8 kb *Apal*/*EcoRI* restriction fragment from GenBank Accession number  
L21902.

13. The recombinant vector of claim 11, further defined as vector pSKSE5.3, wherein:  
the sequence encoding a polyhydroxyalkanoic acid synthase protein is contained within  
the 3.5 kb *SmaI*/*Apal* restriction fragment from GenBank Accession number  
J05003; and

the sequence encoding a fatty acid:acyl-CoA transferase protein is contained within the  
1.8 kb *Apal*/*EcoRI* restriction fragment from GenBank Accession number  
L21902.

14. A recombinant vector comprising:

- a) an isolated nucleic acid segment encoding a polyhydroxyalkanoic acid  
synthase protein;
- b) an isolated nucleic acid segment encoding a fatty acid:acyl-CoA transferase  
protein; and
- c) promoter heterologous to the nucleic acid segment encoding a  
polyhydroxyalkanoic acid synthase protein, and heterologous to the nucleic  
acid segment encoding a fatty acid:acyl-CoA transferase protein.

15. A cell comprising an isolated nucleic acid segment, the nucleic acid segment encoding:

- a) a polyhydroxyalkanoic acid synthase protein; and
- b) a fatty acid:acyl-CoA transferase protein.

16. The cell of claim 15, further defined as a plant cell, mammalian cell, insect cell, fungal  
cell, or bacterial cell.

17. The cell of claim 16, wherein the cell is a plant cell.

18. The cell of claim 16, wherein the cell is a bacterial cell.

19. The bacterial cell of claim 18, wherein the bacterial cell is *Escherichia coli*.

20. The bacterial cell of claim 19, wherein the bacterial cell is *Escherichia coli* strain  
XL1-Blue.

21. The cell of claim 15, wherein the polyhydroxyalkanoic acid synthase protein is a  
*Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein.

22. The cell of claim 21, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid  
synthase protein is encoded by the *Alcaligenes eutrophus phaC* polyhydroxyalkanoic  
acid synthase structural gene.

23. The cell of claim 15, wherein the fatty acid:acyl-CoA transferase protein is a 4-  
hydroxybutyrate:acyl-CoA transferase protein.

AMENDED SHEET

24. The cell of claim 23, wherein the 4-hydroxybutyrate:acyl-CoA transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein.
25. The cell of claim 24, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein is encoded by the *Clostridium kluyveri orfZ* 4-hydroxybutyrate:acyl-CoA transferase structural gene.
26. A method for preparing a transformed cell, comprising the steps:
- selecting a host cell;
  - contacting the host cell and an isolated nucleic acid segment, the nucleic acid segment encoding:
    - a polyhydroxyalkanoic acid synthase protein; and
    - a fatty acid:acyl-CoA transferase protein;under conditions suitable for uptake of the nucleic acid segment by the host cell; and
  - regenerating the cell to produce a transformed cell;
- wherein the nucleic acid segment is free of total genomic DNA
27. The method of claim 26, wherein the contacting step is further defined as calcium chloride mediated transformation.
28. The method of claim 26, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.
29. The method of claim 28, wherein the cell is a plant cell.
30. The method of claim 28, wherein the cell is a bacterial cell.
31. The method of claim 30, wherein the bacterial cell is *Escherichia coli*.
32. The method of claim 31, wherein the bacterial cell is *Escherichia coli* strain XL1-Blue.
33. The method of claim 26, wherein the polyhydroxyalkanoic acid synthase protein is an *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein.
34. The method of claim 33, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus phaC* polyhydroxyalkanoic acid synthase structural gene.
35. The method of claim 26, wherein the fatty acid:acyl-CoA transferase protein is a 4-hydroxybutyrate:acyl-CoA transferase protein.
36. The method of claim 35, wherein the 4-hydroxybutyrate:acyl-CoA transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein.

AMENDED SHEET

37. The method of claim 36, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein is encoded by the *Clostridium kluyveri* *orfZ* 4-hydroxybutyrate:acyl-CoA transferase structural gene.
38. A method for the preparation of a polyester, comprising the steps of:
- 5 a) obtaining a cell capable of producing:
- i) a polyhydroxyalkanoic acid synthase protein; and
- ii) a fatty acid:acyl-CoA transferase protein;
- b) establishing a culture of the cell;
- c) culturing the cell under conditions suitable for the production of the polyester;
- 10 and
- d) isolating the polyester from the cell.
39. The method of claim 38, wherein the cell is a plant cell, mammalian cell, insect cell, fungal cell, or bacterial cell.
40. The method of claim 39, wherein the cell is a plant cell.
- 15 41. The method of claim 39, wherein the cell is a bacterial cell.
42. The method of claim 41, wherein the bacterial cell is *Escherichia coli*.
43. The method of claim 42, wherein the bacterial cell is *Escherichia coli* strain XL1-Blue.
44. The method of claim 38, wherein the polyhydroxyalkanoic acid synthase protein is a polyhydroxyalkanoic acid synthase protein from *Alcaligenes eutrophus*.
- 20 45. The method of claim 44, wherein the *Alcaligenes eutrophus* polyhydroxyalkanoic acid synthase protein is encoded by the *Alcaligenes eutrophus* *phaC* polyhydroxyalkanoic acid synthase structural gene.
46. The method of claim 38, wherein the fatty acid:acyl-CoA transferase protein is a 4-hydroxybutyrate:acyl-CoA transferase protein.
- 25 47. The method of claim 46, wherein the 4-hydroxybutyrate:acyl-CoA transferase protein is a *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein.
48. ~~The method of claim 47, wherein the *Clostridium kluyveri* 4-hydroxybutyrate:acyl-CoA transferase protein is encoded by the *Clostridium kluyveri* *orfZ* 4-hydroxybutyrate:acyl-CoA transferase structural gene.~~
- 30 49. The method of claim 38, wherein the culture contains glucose.
50. The method of claim 38, wherein the culture contains 4-hydroxybutyric acid, the sodium salt of 4-hydroxybutyric acid,  $\gamma$ -butyrolactone, 1,4-butanediol, 4-hydroxyvaleric acid,  $\gamma$ -valerolactone, 1,4-pentanediol, 3-hydroxybutyric acid, the sodium salt of 3-hydroxybutyric acid, a hydroxypropionic acid, a hydroxybutyric acid,
- 35

a hydroxyvaleric acid, a hydroxycaproic acid, a hydroxyheptanoic acid, a hydroxyoctanoic acid, a hydroxydecanoic acid,  $\gamma$ -caprolactone,  $\gamma$ -heptanolactone,  $\gamma$ -octanolactone, or  $\gamma$ -decanolactone.

51. The method of claim 38, wherein the culture contains molecular oxygen.

5 52. The method of claim 38, wherein the cell is further capable of producing a protein capable of hydrolysing a lactone to the corresponding hydroxyalkanoic acid.

53. The method of claim 38, wherein the cell is further capable of producing a 2-oxoglutarate decarboxylase protein and a 4-hydroxybutyrate dehydrogenase protein.

54. The method of claim 38, wherein the cell is further capable of producing a succinate:acetyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

10

sub  
B3

55. The method of claim 38, wherein the cell is further capable of producing a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

15

56. The method of claim 38, wherein the cell is further capable of producing a 2-methylcitrate synthase protein, a 2-methylcitrate dehydratase protein, a 2-methylisocitrate dehydratase protein, a 2-methylisocitrate lyase protein, a succinate:acetyl-CoA transferase protein, a succinate-semialdehyde dehydrogenase protein, and a 4-hydroxybutyrate dehydrogenase protein.

57. The method of claim 38, wherein the polyester is a homopolyester.

20 58. The method of claim 57, wherein the homopolyester is poly(4-hydroxybutyric acid).

59. The method of claim 57, wherein the homopolyester is poly(3-hydroxybutyric acid).

60. The method of claim 38, wherein the polyester is a copolyester.

61. The method of claim 60, wherein the copolyester is poly(3-hydroxybutyric acid-co-4-hydroxybutyric acid).

25

add  
A2

add  
B4